UROONCOLOGY
Review

PSA, PSA derivatives, proPSA and prostate health index in the diagnosis of prostate cancer

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ABSTRACT

Currently, prostate- specific antigen (PSA) is the most common oncological marker used for prostate cancer screening. However, high levels of PSA in benign prostatic hyperplasia and prostatitis decrease the specificity of PSA as a cancer marker. To increase the specificity of PSA, PSA derivatives and PSA kinetics have been used. However, these new techniques were not able to increase the diagnostic specificity for prostate cancer.Therefore, the search for new molecules and derivatives of PSA continues. With the aim of increasing the specificity of prostate cancer diagnosis, proPSA and the Prostate Health Index have been introduced. In this review, the roles of PSA, PSA derivatives, proPSA and the Prostate Health Index in Prostate Cancer diagnosis are examined.

Key words: Prostate cancer; PSA; proPSA; prostate health index.

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Prostate-specific antigen (PSA) belongs to a family of human kallikrein gene family. It is localized at locus q13.2-q13.4 on the long arm of human chromosome 19. PSA which has a serine protease activity is known as human kallikrein-3 with a specific weight of 34kDa.[1] PSA is produced from cells of the columnar epithelium of the prostate tissue. This produced PSA, passes through basal cell layer, smooth muscle, fibroblasts, capillary membrane, and endothelial cells, and enters into systemic circulation.[2] It exerts an effect on semenogelin, and fibronectin proteins which give the seminal fluid gel consistency with resultant liquefication of the seminal fluid. PSA is synthetized as preproPSA which consists of 261 amino acids. A precursor sequence of 17 amino acids dissociate to form a proPSA consisting of 244 amino acids. From proPSA, human kallikrein 2 (hK2), and human kallikre-in 4 (hK4) are removed to form mature, active PSA with 237 amino acids.[3]

Nowadays, prostate-specific antigen is the best first-step serum marker as a screening test for prostate cancer (PCa). It is still the most frequently used oncological marker. It is predominantly found in prostate tissue, and seminal fluid, however its secretion from salivary glands, pancreas, and breast tissue has been reported.[4] Since total PSA has an inadequate potency, and specificity, it is an inadequate diagnostic tool for PCa. PSA levels increase in benign prostatic hyperplasia (BPH), and prostatitis. Therefore in order to compensate for this deficiency, and increase specificity of PSA, various PSA-related serum markers, and PSA derivatives have been used. Despite all of these attempts, a test which definitively establishes diagnosis of prostate cancer is lacking. Relevant attempts to increase specificity, and sensitivity of PSA are still continuing

In this article, all available evaluations of PSA were reviewed in order to avoid unnecesary, and excessive number of biopsies, determine patients eligible for prostatic biopsy, make an early diagnosis, and start treatment at the early phase of the disease. The aim of this review article is to analyze PSA, and PSA derivatives which will play a role in the detection of prostate cancer, and also examine proPSA, and prostate health index which have started to gain popularity in recent years with an integrated approach and due emphasis on this issue.

PSA and PSA derivatives

Total PSA (tPSA)
Prostate-specific antigen was firstly demonstrated in the sera of PCa patients in 1979, and

since then it has been the subject matter of increasing number of investigations. Nowadays, it has been introduced into routine daily practice. Measurement of PSA levels has been recommended for men as young as 40 years of age.[5,6] Total PSA detectable in serum, consists of proteolytically active free PSA, and alpha-1 antichymotrypsin- bound PSA..[7] Priorly performed studies accepted 0-4 ng/mL as a normal range for tPSA. However in 20-25% of the cases with PCa, tPSA values are lower than 4 ng/mL. Even a PCPT (Prostate Cancer Prevention Trial) study detected tPSA levels lower than 0.5 ng/mL in 20-25% of the patients with PCa.[8,9] In 25, and even 67% of the patients with PCa, respective tPSA values of 4-10 ng/mL, and above 10 ng/mL were observed.[10] A tPSA value of 4.0 ng/mL has 20% sensitivity, and 60-70% specificity.[11] Therefore, investigators have started to use PSA derivatives, and PSA kinetics in the prediction of risk, and aggressiveness of PCa hoping to overcome these inconveniences.

Free PSA (fPSA) and Free/Total PSA (f/tPSA) ratio
Free form constitutes 5-40% of total PSA. In serum, fPSA does not form complexes with antiproteases in serum, and they are found in unbound forms in blood. Ordinarily, it has no diagnostic value *per se*. It is used in the f/tPSA ratio formula which is expressed as % fPSA=100 x fPSA/tPSA. Lower % fPSA has been associated with prostate cancer, and many studies indicated its value in reducing number of negative biopsies.[13] However in recent studies 3 forms of fPSA have been observed in serum. One of them is inactive PSA. It is known as proPSA or PSA precursor. The other two forms are BPSA (Nicked PSA), and intact PSA (iPSA).[12] In a meta-analysis published in 2005 demonstrated that % fPSA was helpful in the increasing detection of PCa.[13] Later on, subforms of fPSA including proPSA, iPSA, and BPSA have been found. BPSA, and iPSA have been associated with benign tissue, while proPSA was found in prostate cancer tissue.[14] Especially in men with PSA levels ranging between 4, and 10 ng/mL, f/tPSA is more sensitivity in discriminating PCa from benign tissues.[15] In a prospective multicentered study % f/tPSA values of <10%, and >25% could detect PCa in 56, and 8% of the patients, respectively.[16]

Complexed PSA (cPSA)
In the serum, cPSA is found in a conjugated form, and constitutes 60-95% of total PSA. A 60-90% of cPSA is bound to antichymotrypsin (ACT), 10-20% to alpha 2 macroglobulin (AMG), and 1-5% to alpha -1 protease inhibitor (API). PSA-AMG undergoes hepatic clearance within 6-7 minutes, while half-life of PSA-ACT complex is 3 days. ACT, AMG, and API complexes form cPSA.[17]

BPSA ve Nicked PSA
Steuber et al.[18] used different methods for the detection of internally broken multichain serum fPSA. In their measure-

ments they used nicked PSA (sPSA-N) which is obtained by extraction of a single-chain sPSA forms. Both serum BPSA, and sPSA-N correlate with benign prostatic hyperplasia (BPH). It has been demonstrated to be more significant than other PSA forms.

Intact PSA
It is another subfraction of PSA. Since it is isolated from LNCaP cells, its place in clinical practice is still debatable.[19]

PSA Density (PSAD)
Prostate volume evaluated by transrectal US is defined as tPSA ratio. The contributions of normal prostate epithelium, BPH or PCa tissue to serum tPSA levels have been estimated to be 0.1 ng/mL, 0.3 ng/mL, and 3.5 ng/mL, respectively.[20] Since, transrectal US is device-, and physician-dependent, and because of interdividual differences between stroma/epithelium ratios, it has a limited use.[21] Catalona et al.[22] demonstrated that when they accepted a lower PSAD cut-off value of 0.1, re-biopsies could be reduced by 31% with a sensitivity of 90 percent. However, sPSA values can also achieve this level of performance Therefore, nowadays it has no place in clinical practice, but it is used in academic investigations.

PSA velocity
Total PSA velocity (ng/mL/year) has been defined as annual absolute increase in tPSA. In patients with serum PSA levels between 4, and 10 ng/mL, it is defined as annual increase of 0.75 ng/mL when compared with the baseline value. However later on, increases of 0.35 ng/mL in PSA velocity have been associated with PCa mortality.[23,24] Nowadays, an accepted threshold value for PSA has not been determined yet.

PSADT (PSA doubling time)
It is defined as the number of months it takes before a baseline PSA value doubles. It is calculated by the formula [log(2)*T2-T1(time difference)]/[log PSA2-logPSA1] It is considered to be more important in stages of treatment, and recurrences, rather than diagnosis.[25]

Age- specific PSA
In recent studies an age-related logarithmic correlation was observed between serum tPSA value, and prostate volume. In young patients, it has raised diagnostic sensitivity considerably, and increased the number of biopsies at a rate of 45 percent. On the other hand, age-specific PSA has reportedly increased the detection rate of clinical PCa 20-60% in individuals over 60 years of age. Therefore, it is not a significant parameter by itself.[26,27]

Total PSA interval between 2 and 4 ng/mL
PCa detection rates in men with PSA values within this interval have been reported in many studies. In the PCPT study, in the

control group with PSA levels lower than 4 ng/mL, occult, and high grade PCa were detected in 15, and 14% of the patients, respectively. Detection rates of PCA were found to be 10, 17, 24, and 27% in patients with tPSA levels <0.5, 0.5-1.0, 1.1-2.0, 2.1-3, and 3.1-4 ng/mL, respectively. Since based on these results, in patients with tPSA levels between 2, and 4 ng/mL clinically significant PCa incidence rates have been detected, time-adjusted tPSA values have become significant. Punglia et al.[28], reported that when they used tPSA cut-off values of 2.6 ng/mL instead of 4.1 ng/mL, PCa detection rates had risen from 18 to 36 percent. They also added that when tPSA cut-off value of 4.0 ng/mL was used for screening, 84% of the cases with PCa might be overlooked.

ProPSA (pPSA)

It is a precursor of prostate-specific antigen which contains 244 amino acids. It is released from peripheral cancerous tissue rather than transitional hyperplastic tissue. It is found both in tissue, and serum. It is also abundant in malignant cells, and higher grade intraepithelial neoplasias (H-PIN). Frequently, it is defined as [-7]pPSA. However, some recent publications asserted that this pPSA has also some subfractions.[29] Its another form is [-2]pPSA (p2PSA) described by Mikolajczyk et al.[30] which contains 239 amino acids. It is firstly found in PCa tissue, and then in sera. Peter et al.[31] used a different approach to obtain diverse forms of pPSA in the sera of the patients with PCa. Among them [-7], and [-5] pPSA forms were detected in all patients investigated, however [-2], and [-1] pPSA forms were found only in three patients. While [-6], and [-4] pPSA forms were not observed in the sera of the patients. Whereas, p2PSA was the most stable form in serum.[14]

In many studies related to proPSA, rates for tPSA/pPSA, pPSA/sPSA, pPSA/sPSA/tPSA, and pPSA/BPSA have been determined. For example, Khan et al.[32] indicated that in cases with tPSA values ranging between 4, and 10 ng/mL, pPSA levels measured in order to refrain from unnecessary biopsies had 90% sensitivity, and 44% specificity. Some other authors reported sensitivity rates of 33, and 75% for f/tPSA, and tPSA/pPSA, respectively.[12] Other studies evaluated use of pPSA, and BPSA in patients with f/tPSA ratios of ≤15%, and found 90% sensitivity, and 46% specificity for pPSA/BPSA in patients with tPSA levels varying between 1.8, and 24 ng/mL.[32] Miyakubo et al.[12], evaluated correlations among f/tPSA, pPSA/tPSA, pPSA/sPSA, pPSA/fPSA/tPSA, PSAD, and PSATZD, in addition to Gleason scores (GS) in 257 biopsy specimens of patients with tPSA values of 4.1-20 ng/mL. They reported that compared with healthy individuals, f/tPSA ratios were significantly different in patients with PSA values of 4.1-10 ng/mL. They also noted that pPSA/fPSA, and pPSA/fPSA/tPSA ratios, PSAD, and PSATZD values were significantly higher in PCa patients. These values climbed to even higher levels in cases with GS ≥7. Catalona et al.[33]

stated that these ratios would have predictive value for PCa. Within this context, p2PSA was reported as the best marker. Histopathological studies have revealed that clinically aggressive cancers could be detected by using this marker. However, Stephan et al.[34] demonstrated that [-5,-7] pPSA values were significantly different in a subgroup of patients with pT2, and pT3 whose f/t PSA levels were below 10 percent. pPSA/fPSA/tPSA ratios demonstrated differences between patients with GS <7, and GS ≥7, and also pT2 , and pT3, however in patients with PSA values between 10.1, and 20.0 ng/mL, and a strong correlation was shown between GS 8-10, and abovementioned ratios. Miyakubo et al.[12] indicated that pPSA/fPSA ratio increased detection rate of PCa in patients with Gleason score of 6. Therefore they stated that especially in young patients with a longer life expectancy, pPSA might be a very helpful predictive marker before development of serious complications. In combination with GS component, pPSA/fPSA, and pPSA/fPSA/tPSA ratios are important markers in the definition of PCa risk in young men with long life expectancies before initiation of definitive therapy.

However on the other hand, Bengma et al.[29] used [-7,-5] pPSA, hK2, and sPSA values in patients with higher tPSA in order to discriminate between PCa, and BPH. However they couldn't demonstrate existence of any correlation between combinations of pPSA, and diagnosis, and grade of the tumor, and pointed out to their limited effectiveness. Whereas, they emphasized that p2PSA is a more sophisticated discriminative marker. While, among 119 men, in those with tPSA levels between 2.5, and 4 ng/mL, pPSA /tPSA increased diagnostic accuracy of detecting PCa. Use of percent pPSA significantly decreased the number of unnecessary biopsies even in small sample- sized studies.[35]

In 1091 men, pPSA/tPSA ratio was compared to % fPSA. In men with tPSA levels between 2-4 ng/mL (n=555), biopsy pathology results were compared with % pPSA, and % pPSA identified PCa with 90% accuracy. Within a lower tPSA interval, % pPSA is statistically significantly different from % fPSA. It has been demonstrated that in cases with GS over 7, pPSA were significantly higher which might indicate aggresiveness of the tumor. In PCa patients with GS >7), especially pPSA was 50% higher when compared with those with lower Gleason scores (GS <6). As an outcome of this study, pPSA provides us an opportunity to detect prostate cancers at an early stage, institute more aggressive treatment or prefer watchful waiting.[35,36]

Improvement in the application of p2PSA tests using Beckman Coulter device has opened new fields of investigation in the detection of prostate cancer. In a few studies performed in patients with tPSA levels of 2.5- 10 ng/mL, in the discrimination between PCa, and BPH, p2PSA/fPSA ratio was reportedly more meaningful as for detection of PCa relative to tPSA or % fPSA.[14]

Naya et al.[37], indicated lack of any significant difference between patients with or without PCa as for pPSA values, and % pPSA within a tPSA interval 4.4-8.5 ng/mL. Catalona et al.[38] observed increases in % pPSA values in PCa patients with tPSA values varying between 2, and 10 ng/mL, and stated that % pPSA has higher diagnostic sensitivity, and specificity for PCa when compared with % fPSA without any correlation between histopathological stage, and % pPSA. Mikolajczyk et al.[39] also pointed out to higher specificity, and positive predictive value of % pPSA rather than % fPSA for PCa in cases with tPSA values between 4, and 10 ng/mL. While de Viries et al.[40] reported that at tPSA values below 15 ng/mL, % pPSA did not correlate with prognosis of PCa patients, and they also expressed that higher % pPSA values at tPSA interval of 4-10 ng/mL were associated with poor prognosis.

Human Kallikrein-2 (hK2)
PSA in prostatic tissue namely [-7] pPSA, is converted to mature PSA via activation of hK2 induced by a physiological process occurring in seminal fluid. Human kallikrein 2, is a protein belonging to a kallikrein family closely related to PSA.[41] In prostate cancer various precursors of hK2 have been detected.[42] Theoretically, hK2 is related with PSA levels, and conjugated forms are found both in tissue, and serum. It is a potential tumor marker. In a previous study, hK2 demonstrated its usefulness in the discrimination between prostate cancer, and BPH. Therefore, hK2, and hK2 tPSA /fPSA values could be used for the differentiation between pathological grades of the tumor. Pivot studies have displayed its usefulness as a volumetric marker of the prostatic tissue.[43,44]

ProPSA, and Prostate Health Index (PHI)
It is a mathematical equation demonstrating probability of prostate cancer This equation is formulated as p2PSA/fPSA)/√tPSA [14] Higher PHI values have been associated with increased probability of prostate cancer. Le et al.[45] performed ROC analyses, and demonstrated the highest performance for phi among other tumor markers with an AUC of 0.77. With a specificity of 33%, phi reached nearly 2-fold specificity of fPSA/tPSA. In ROC analysis phi has the highest AUC value of 0.72 which is extremely different from % fPSA with a p value of 0.0001.[46]

In a meta-analysis of 12 studies, for % p2PSA, the degree of specificity, and sensitivity comparable to those of phi was tried to be estimated. However only in three of these studies cut-off values could be used. In some studies non-commercial kits of p2PSA were used. When this meta-analysis which mostly contained heterogenous groups was analyzed, at a clinically acceptable level of sensitivity (90%), degrees of specificity for p2PSA, and phi were 32% (21%-49%), and 32% (26-43%), respectively. AUCs estimated for p2PSA, and phi were 0.635-0.780, and 0.703-0.77, respectively. Degrees of specificity were

89, and 81% for p2PSA, and phi, respectively.[14,39,47-49] In this meta-analysis, in patients with tPSA levels between 2-4, and 4-10 ng/mL, % p2PSA, and phi appeared to have comparable performances.[14] In most of the studies, AUC of % fPSA was higher than that of % p2PSA.[14] However, Sokoll et al.[49] demonstrated higher AUC values for % p2PSA when compared to fPSA in patient groups with tPSA levels between 2, and 10 ng/mL. This outcome underlines potential usefulness of % p2PSA in the detection of prostate cancer in patients with tPSA values ranging between 2, and 2-10 ng/mL.[49] In this meta-analysis, tests of phi derivatives appear to display a comparable to or slightly better performance than % p2PSA tests.[14] In various studies performed, with artificial neural network (ANA) or logistic regression tests have demonstrated that performance of PSA derivatives were better than that of p2PSA. The best results for AUC (0.85-0.84). have been presented by Stephan et al.[34] using ANN, and logistic regression models. Age-adjusted artificial neural network which includes % p2PSA, % fPSA, tPSA, and % fPSA or % p2PSA achieved a higher level of significance at 90, and 95% sensitivity with a 17-28% increase in specificity.

Although the above-mentioned results demonstrate that % p2PSA, and phi have an increased specificity for the detection of prostate cancer which decreases number of unnecessary biopsies, cut-off values of these tests have not been indicated in most of the relevant articles. Cut-off values for % p2PSA with a 90% sensitivity were reported as 2.5% (Mikolajczyk et al.[39]), and 1.06 % (Miyakubo et al.[47]), while comparable cut-off values of phi with also 90% sensitivity were reported by Miyakubo et al.[47] (24.5%), and Catalona et al.[48] (21.1%) as indicated in parentheses. Catalona indicated that[48], PHI does not change with age, and so it can be easily used to detect PCa in youngsters, and the elderly.

Analysis of cost-effectiveness has demonstrated that p2PSA is 2-3 times more costly than both tPSA, and fPSA, however in the detection of PCa, %p2PSA, and phi decreases global expenditures. Cost of additional blood tests compensate for unnecessary biopsies refrained from, and physicians'fee.[14]

A potential association has been demonstrated between % p2PSA, phi and aggressiveness of PCa, and also their higher levels in patients with GS ≥7, and those with locally advanced disease.[14,49] Some recent data have indicated that one third of the newly diagnosed tumors are not apparently in their advanced stages[50], and thus these patients are candidates for active surveillance. However standard markers, tPSA, biopsy results, GS, and the number of positive cores fall short of identification of these patients for more appropriate selection of treatment, and more accurate prediction of aggressiveness of prostate cancer in these patients. This finding was confirmed by PIVOT study which compared watchful waiting with radical prostatectomy in 731

patients with localized PCa, and the authors stated that radical prostatectomy increased survival rates in patients with moderate, and high-risk patients with tPSA levels over 10 ng/mL.[51]

In a multivariate analysis, Guazzoni et al.[52] demonstrated that in the prediction of higher Gleason score or pathological stage, combined use of % p2PSA, and phi increased accuracy rate of prediction in a model based on combination of biopsy GS, and clinical stage, % sPSA, tPSA, and age of the patients. Similarly, de Vries et al.[40] used Epstein criteria to differentiate between aggressive, and non-aggressive tumors, and stated that % p2PSA test results provide promising guidelines in the selection of treatment strategies for PCa patients Finally, in a study by Isharwal et al.[53] use of % p2PSA, and phi decreased the number of biopsies in patients on active surveillance. Based on the results obtained, % p2PSA, and ve phi can make a discrimination between high-, and low-risk prostate cancers.

In a multicentered European study Lazzeri et al.[54] demonstrated that in patients with tPSA 2-10 ng/mL, p2PSA derivatives increased the prediction rates for PCa. In their study, prebiopsy serum tPSA, fPSA, and p2PSA values were compared with biopsy results.In the assessments of high degree of sensitivity % fPSA, of best combination % p2PSA, phi, and sPSA, of higher specificity % p2PSA, and phi had taken the first place among all parameters namely both sensitivity, and specificity,and also positive, and negative predictive values. Besides, % fPSA ranked on top in avoidance from unnecessary biopsies (57.4% of the cases), and detection of PCa (43.9% of the cases). In the analysis of ROC of the patients with GS≥7 disease, phi (AUC: 0.65), % p2PSA (AUC: 0.64), and % fPSA (AUC: 0.59) were the top three markers most predictive for PCa. In conclusion, in patients with tPSA values between 2, and 10 ng/mL the most robust parameters predicting PCa in baseline biopsy samples were shown to be % p2PSA, and phi.

In conclusion, total PSA, % fPSA, f/tPSA, and other PSA derivatives, PSAD, PSA velocity, PSADT, age-specific PSA do not decrease the number of unnecessary biopsies performed for diagnostic purposes. Besides, chaos still continues in that during active monitorization some inconveniences might be experienced, and lower grade PCa's might be overestimated. Investigations continues at full speed so as to prevent this state of confusion, decrease the number of unnecessary biopsies, and establish the diagnosis at an early stage of the disease. One of these promising studies is related to pPSA, pPSA derivatives, and phi. Excluding some of these studies on this subject, the relevant investigations have been demonstrated to decrease the number of unnecesary biopsies, and the patients under active surveillance. Besides these parameters have shown parallelism with Gleason scores with increased efficacy in detecting aggressive tumors. Despite all of these favorable factors, a 100% conclusively precise diagnostic test for PCa has not been introduced yet.

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